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Public Health Implications of Antibiotic-Resistant Bacteria Associated with Suya Spices in Nigeria

Temitope Kudirat Bello; PhD [1] & Olorunjuwon Omolaja Bello; PhD *[2]

[1] *Department of Biological Sciences, Elizade University, Ilara-Mokin, Ondo State, Nigeria.*
[2] *Department of Biological Sciences, University of Medical Sciences, Ondo City, Ondo State, Nigeria.*

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****Corresponding author:***
obello@unimed.edu.ng
Department of Biological Sciences, University of Medical Sciences, Ondo City, Ondo State, Nigeria.

Postal code: 351104
Tel: +2348057892661

ABSTRACT

Background: The emergence and re-emergence of antibiotic-resistant foodborne bacteria call for concerted efforts, especially in developing countries. Spices have been employed traditionally to improve the aroma and flavour of foods; however, they could serve as reservoirs for the spread of potential pathogenic organisms. This study aims to investigate the public health implications of antibiotic-resistant bacteria associated with suya spices. **Methods:** Three hundred samples of suya spices were evaluated for bacteriological quality. The isolates were presumptively identified by standard biochemical tests and confirmed by API 20 E and API 20 NE. The Etest was used for determining the minimum inhibitory concentration (MIC) of the drugs, while the antibiotic resistance profile of the isolates was evaluated using the Kirby Bauer methods. **Results:** The mean total plate count (TPC) ranged from 1.0 x 10^3 to 1.6 x 10^3 CFU/g. The TPCs of control samples showed significant differences in various zones ($P < 0.05$). One hundred and thirty-three bacterial isolates were obtained from suya spices. Pseudomonas aeruginosa (25.56%) had the highest frequency and Klebsiella planticola (3.01%) the lowest. The MIC of antibiotics ranged from 0.02 mg/l to > 256. The percentage of isolates resistant to the antibiotics ranged from 3.01% to 87.97% as exhibited by imipenem and teicoplanin, respectively. **Conclusion:** This study revealed a high bacterial load and the presence of potential pathogens in suya spices. This depicts that suya spices, when added to suya meat, are one of the sources of microbial contamination.

Keywords: *Anitibiotic resistance; Foodborne pathogens; Food safety; Hygiene; Microbiological quality; Spice*

Introduction

Suya is a ready-to-eat spicy meat product. It is usually roasted, smoked or barbecued. The Hausas from the northern part of Nigeria rear cattles as a means of livelihood and are well-known for suya preparation. The thinly spliced meat are marinated by spices, and then barbecued. There is no standard recipe for the production of complex mixture of additives and spices that make up suya marinade (Bello Temitope and Bello Olorunjuwon, 2020, Orogu and Oshilim, 2017).

The terms ‘herbs’ and ‘spices’ which are frequently used interchangeably, are plant-derived

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seasonings used for culinary purposes. These substances, however, have their typical and denite descriptions. Herbs usually store their aromatic components in leaves, while the flavor and aromatic components of spices are stored in roots, seeds, and bark. A typical spice may include the bark (cinnamon), root (ginger), aromatic seed (cumin), bud (clove), and flower stigma (saffron) of a plant (Ogbunugafor *et al.*, 2017). Apart from the enhancement of the quality of taste, spices have been considered for their health-enhancing characteristics and also employed as food preservatives.

Spices have been used, not only as flavouring agents and antioxidants, but for their antimicrobial activities against foodborne pathogens. Despite the beneficial qualities spices confer on food, they could harbor potential bacterial pathogens and pose a public health risk to consumers. The possible sources by which spices get contaminated include unhealthy processing and storage equipment, poor handling techniques, unhygienic display environment, vehicular transmission, exposure to atmospheric particles and air-borne microorganisms (Bakobie *et al.*, 2017).

The safety of ready-to-eat food is of major concern, not only to the consumers but also the producers. Despite the high level of awareness on food processing and preservation methods, there is increasing occurrence of foodborne disease outbreaks caused by pathogenic and spoilage microorganisms in foods (Barber *et al.*, 2018). Suya spices are naturally prepared in combination with selected medicinal plants and the methods of preparation lack good hygiene practices. Studies carried out by some researchers showed that spices contain potential pathogens of public health significance which could give rise to food poisoning outbreak (Amare *et al.*, 2019, Bakobie *et al.*, 2017, Odu and Akwasiam, 2016). Unfortunately, many of these potential pathogens have evolved resistance mechanisms to usual selected antibiotics.

Antibiotic resistance is of profound public health concern and rapid detection of the resistant bacterial strains is essential for curbing the spread of resistance and control disease conditions. When the antibiotic does not kill or inhibit a pathogenic organism, it is interpreted as dosage failure or drug tolerance. When an organism exhibits resistance to two or classes of antibiotics, it is said to be multidrug-resistant. Several resistant mechanisms may be found in bacterial strains (Shaikh *et al.*, 2015). This study aims to investigate the public health implications of antibiotic-resistant bacteria associated with suya spices in Nigeria.

Materials and Methods

Collection of suya spices samples: A total of three hundred suya spice samples were analyzed in this study. Two hundred and forty suya spices samples were bought from different locations in four geopolitical zones (Yewa, Egba, Remo, and Ijebu zones) of Ogun State, Nigeria. The samples were collected and kept in a sterile air-tight container (as samples were purchased at night) and then transported to the laboratory for microbial analyses. Sixty suya spice ingredients, purchased raw from different locations and zones under study, were prepared to serve as the control. For the purpose of this study, Yewa, Egba, Remo, and Ijebu zones were labeled zones A, B, C, and D, respectively, while the samples prepared in the laboratory served as control.

Preparation of suya spices ingredients: The ingredients normally used as suya spices, which are also known as yaji, were purchased from specialized spice market in Ogun State, Nigeria. The spice ingredients included ginger (*Zingiber officinale)*, cloves (*Syzygium aromaticum*), alligator pepper (*Afromomum melegueta*), black pepper (*Piper guineense*), Nutmeg (*Myristica fragans*), Africa negro pepper (*Xylopia aethiopica*), and red pepper (*Capsicum fructescens*). Other constituents of the ingredients were groundnut cake powder (*Arachis hypogea*), salt (*Sodium chloride*), and seasoning (*Monosodium glutamate*). The spices and other ingredient constituents were washed with distilled water and further dried using the hot-air oven at 60 ^{o}C for 72 hrs. All ingredients were milled individually and mixed together in a specific proportion as described by (Apata *et al.*, 2013)

(Apata *et al*., 2013). The suya spices, prepared under controlled environment, served as the control.

Determination of microbial load of suya spices: The microbiological analysis of suya spices was carried out as described by (Splittstoesser and Vanderzant, 1992) and Association of Official Analytical Chemist (AOAC, 2000). A 10-gram portion of suya spices was measured into 90 ml of 0.1% (W/V) peptone water for 60 sec. Additional dilutions were made in 0.1% peptone water (W/V). Serial dilutions were made to obtain up to 10^{-5} dilution factor and 0.1 ml portion of each of the diluted samples was taken and dispensed in sterile Petri dishes containing appropriate agar media using the spread plate method. Total bacterial count was determined on plate count agar (Difco, USA), and total coliform on violet red bile agar (Difco, USA). Further differentiation of specific coliform organisms was determined by IMViC tests. Eosin methylene blue agar, tellurite agar and mannitol salt agar (Oxoid, England) were used for the cultivation of *Escherichia coli*, *Enterobacter* species, and staphylococci, respectively. All plates were incubated at 37 °C for 24 - 48 hrs and colony forming units were expressed in log10 cfu/g of samples.

Identification of isolates: Pure isolates were obtained by continuous subculturing, and characterization of pure isolates was achieved by initial morphological examination for colonial appearance, size, odour, opacity, edge, consistency, elevation, form, colour, pigmentation, and haemolysis. Biochemical tests were then carried out on the isolates in accordance to standard procedures. Additional identification and confirmation of members of Enterobacteriaceae and non-Enterobacteriaceae was achieved using API 20E and API 20NE, respectively (Bello Temitope and Bello Olorunjuwon, 2020).

Antibiotic sensitivity profiling using the etest: This was carried out as described in (Bello Temitope and Bello Olorunjuwon, 2020). The investigated antibiotics were tetracycline (0.002–32 mg/l), doxycycline (0.002–32 mg/l), minocycline (0.002–32 mg/l), erythromycin (0.016–256 mg/l), colistin (0.064–1024 mg/l), chloramphenicol (0.016–256 mg/l), trimethoprim/sulfamethoxazole (0.002–32 mg/l), gentamicin (0.016–256 mg/l), rifampicin (0.002–32 mg/l), nalidixic acid (0.016–256 mg/l), ciprofloxacin (0.002–32 mg/l), penicillin G (0.002–32 mg/l), ampicillin (0.016–256 mg/l), imipenem (0.002–32 mg/l), cefalotin (0.016–256 mg/l), ceftriaxone (0.016–256 mg/l), teicoplanin (0.016–256 mg/l), and vancomycin (0.016–256 mg/l). The results were interpreted in line with the (Clinical Laboratory Standards Institute (CLSI), 2016).

Data analysis: The data were analysed statistically with the use of IBM SPSS Statistics, version 25. Simple means, frequencies, and percentage occurrences of microorganisms from various locations were compared with the One-way Analysis of Variance (ANOVA) and students' independent t-test. The mean±standard error (SE) of triplicate data and significant differences were determined at 95% level of confidence (P-value ≤ 0.05).

Results

Mean total bacterial counts from suya spices samples: The mean total plate count (TPC) of suya spices from zones A, B, C, and D ranged from 1.0 x 10^3 to 1.6 x 10^3 CFU/g, 1.1 x 10^3 to 1.4 x 10^3 CFU/g, 1.0 x 10^3 to 1.4 x 10^3 CFU/g, and 1.0 x 10^3 to 1.3 x 10^3, respectively. However, the TPC of control samples ranged from 0.3. x 10^2 to 1.0 x 10^2 CFU/g (**Table 1**). Statistical analyses revealed that there was no significant difference among the TPCs from zones A, B, C, and D ($P > 0.05$), while the TPCs of control samples showed a significant difference with those obtained from each of the zones ($P < 0.05$) (**Table 1**).

Table 2 shows the bacterial species isolated from suya spices in Ogun State, Nigeria. One hundred and thirty-three isolates obtained from the samples of suya spices were characterized into nine bacterial species, including *Serratia rubideae*, *S. epidermidis*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *E. aerogenes*, *K. planticola*, *B. cereus*, and *E. coli*. Out of 133 isolates obtained from the suya spices, 35 isolates, 33 isolates, 24 isolates, and 24

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isolates were encountered from zones A, B, C, and D, respectively. However, 17 isolates were obtained from the control samples.

The occurrence percentage of individual bacterial species from suya spices in different zones: **Figure 1** shows the occurrence percentage of the individual bacterial isolates from suya spices in different geopolitical zones in Ogun State, Nigeria. In zone A, *P. aeruginosa* had the highest occurrence percentage of 25.71%, followed by *S. epidermidis* (20%), *S. aureus* (17.14%), *B. cerues* (14.29%), *S. rubidaea* (11.43%), *E. aerogenes* (5.71%), and *B. subtilis* (5.71%), while *E. coli* and *K. planticola* were not encountered. In zone B, the occurrence percentage of each of *S. aureus* , *S. epidermidis,* and *B. cereus* was 15.15%, while each of *E. coli*, *E. aerogenes*, *P. aeruginosa*, and *K. planticola* was 3.03%. *S. rubidaea* and *B. subtilis* had occurrence percentages of 9.09% and 6.06%, respectively. In zone C, each of *S. rubidaea*, *P. aeruginosa*, and *B. cereus* had percentage occurrence of 16.67%, while each of *E. coli*, *E. aerogenes*, *S. aureus*, and *S. epidermidis* had occurrence percentage of 8.33%. *K. planticola* had occurrence percentage of 4.17%, while *B. subtilis* was not encountered. In zone D, each of *E. coli*, *S. rubidaea*, *P. aeruginosa*, and *S. epidermidis* had occurrence percentage of 16.67%, followed by *S. aureus* (12.5%), *B. cereus* (8.33%), *K. planticola* (8.33%), and *B. subtilis* (4.17%), while *E. aerogenes* was not encountered. In the control samples, *S. aureus* had the highest occurrence percentage of 29.41%, followed by *P. aeruginosa* (23.53%), *S. rubidaea* (17.65%), *S. epidermidis* (11.77%), *B. subtilis* (11.77%), and *E. coli* (5.88%). *E. aerogenes*, *B. cereus*, and *K. planticola* did not occur in the samples.

Occurrence percentage of bacterial species from suya spices: The occurrence percentage of bacterial species in suya spices was shown in **Figure 2**. *P. aeruginosa* had the highest frequency of 25.56%, followed by *S. aureus* (15.79%), *S. epidermidis* (15.04%), *S. rubidaea* (13.53%), *B. cereus* (12.03%), *E. coli* (6.02%), *Bacillus subtilis* (5.26%), *E. aerogenes* (3.76%), and *K. planticola* (3.01%).

The antibiotic susceptibility pattern of bacterial isolates from suya spices: The isolates from suya spices showed varying degrees of sensitivity and resistance to the antibiotics. All strains of *E. coli* encountered in this study showed 100% susceptibility to tetracycline, doxycycline, minocyline, erythromycin, colistin, chloramphenicol, trimethoprim/sulfamethoxazole, and imipenem, while completely resistant to penicillin G, teicoplanin, and vancomycin. *Enterobacter aerogenes* strains exerted 100% susceptibility to tetracycline, doxycycline, minocyline, erythromycin, colistin, trimethoprim/sulfamethoxazole, gentamicin, and imipenem, while fully resistant to rifampicin, penicillin G, teicoplanin, and vancomycin. *Serratia rubidaea* strains showed the maximum susceptibility to tetracycline, doxycycline, minocyline, colistin, chloramphenicol, trimethoprim/sulfamethoxazole, gentamicin, ciprofloxacin, and imipenem, while 100% resistance was shown to teicoplanin and vancomycin. The strains of *P. aeruginosa* encountered in the study showed full susceptibility to only colistin and nalidixic acid, while exhibited complete resistance to rifampicin, penicillin G, ceftriaxone, teicoplanin, and vancomycin (**Table 3**).

Cumulative percentage of susceptibility and resistance profile of bacterial isolates from suya spices: The cumulative percentage of susceptibility and resistance profile of bacterial isolates from suya spices to various antibiotics are shown in **Figure 3**. Considering the 133 bacterial isolates from the suya spices against which the antibiotics were investigated, 94.74% of the isolates were susceptible to imipenem. Minocycline exerted efficacy in 87.21% of isolates, including trimethoprim/sulfamethoxazole (82.70%), doxycycline (79.70%), gentamicin (76.69%), chloramphenicol (69.92%), tetracycline (68.42%), ciprofloxacin (67.67%), ampicillin (63.12%), colistin (60.15%), erythromycin (59.39%), cefalotin (57.14%), ceftriaxone (48.12%), penicillin G

(47.37%), rifampicin (42.10%), nalidixic acid (20.30%), vancomycin (13.53%), and teicoplanin (2.26%). The percentage of isolates resistant to the antibiotics ranged from 3.01% to 87.97% as exhibited by imipenem and teicoplanin, respectively, while those exhibited intermediate susceptibility to the antibiotics ranged from 0 to 9.77%.

Cumulative distribution of susceptible and resistant bacterial strains from suya spices in the different zones: The percentages of resistant bacterial isolates from suya spices in zones A, B, C, and D were 23.53%, 21.25%, 23.55%, and 21.34%, respectively. However, the percentages of susceptibility among the zones were 17.56%, 18.83%, 17.25%, and 17.73%, respectively. The percentages of intermediately susceptible isolates from the suya spices ranged from 21.01% to 21.69. The resistance and susceptibility profiles of bacterial isolates from the four zones showed no statistical difference ($P > 0.05$). However, there was a statiscal difference between the control and the four zones ($P < 0.05$, **Figure 4**).

The findings from this study revealed that suya spices prepared at home, under controlled condition, had the best microbial quality. There was no statistical difference among the level of bacterial contamination in zones A, B, C, and D ($P > 0.05$). The data from these zones, however, showed a significant difference compared to the control ($P <$ 0.05). This indicates the fact that control of contamination of the product can be achieved if aseptic techniques are employed during processing and display. The isolates showed varying degrees of susceptibility to the antibiotics. There was variation in the susceptibility and resistant patterns of the bacterial strains. No statistical difference was observed among the zones ($P > 0.05$), while there was a significant difference between each of the zones and the control ($P < 0.05$, **Figure 4**).

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Table 1. Mean total bacterial counts from suya spices in Ogun State, Nigeria.

Zone	Total plate count (CFU/g)	Total Enterobacteria count (CFU/g)	Total *Staphylococcus* count (CFU/g)
A	1.22×10^{3a}	1.12×10^{2b}	0.24×10^{c}
B	1.19×10^{3a}	1.16×10^{2b}	0.23×10^{c}
C	1.20×10^{3a}	1.25×10^{2b}	0.23×10^{c}
D	1.21×10^{3a}	1.54×10^{3b}	1.14×10^{c}
Control	0.72×10^{2b}	0.05×10^{2a}	0.13×10^{2b}

Each value ($_{1-10}$) represents mean of data from six different suya spots where suya spices were purchased. Means of means with same superscript along same column had no statistical difference.

Table 2. Bacteria isolated from suya spices in Ogun State, Nigeria.

Frauction	No. of isolates	Isolates per zone
Serratia rubidaea	18	A=4,B=3,C=4,D=4,CT=3
S. epidermidis	20	A=7,B=5,C=2,D=4,CT=2
S. aureus	21	A=6,B=5,C=2,D=3.CT=5
P. aeruginosa	34	A=9,B=10,C=7,D=4,CT=4
Bacillus cereus	16	A=5,B=5,C=4,D=2,CT=0
Bacillus subtilis	7	A=2,B=2,C=0,D=1,CT=2
E. aerogenes	5	A=2,B=1,C=2,D=0,CT=0
Escherichia coli	8	A=0,B=1,C=2,D=4,CT=1
K. planticola	4	A=0,B=1,C=1,D=2,CT=0

A: Zone A; B: Zone B; C: Zone C; D: Zone D; and CT: Control

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Table 3. Percentage of antibiotic susceptibility of bacterial isolates from suya spices in Ogun State, Nigeria.

Antibiotic	Status	E. coli n=8	Enterobacter aerogenes n=5	Serratia rubidaea n=18	P. aeruginosa n=34	S. aureus n=21	S. epidermidis n=20	Bacillus cereus n=16	Bacillus subtilis n=7	K. planticola n=4
Tetracycline (0.002–32 mg/l)	S	8 (100)[a]	5 (100)	18 (100)	10 (29.4)	7 (33.3)	20 (100)	12 (75)	7 (100)	4 (100)
	I	0	0	0	0	4 (19.0)	0	0	0	0
	R	0	0	0	24 (70.6)	10 (47.7)	0	4 (25)	0	0
Doxycycline (0.002–32 mg/l)	S	8 (100)	5 (100)	18 (100)	10 (29.4)	18 (85.7)	20 (100)	16 (100)	7 (100)	4 (100)
	I	0	0	0	0	0	0	0	0	0
	R	0	0	0	24 (70.6)	3 (14.3)	0	0	0	0
Minocycline (0.002–32 mg/l)	S	8 (100)	5 (100)	18 (100)	17 (50.0)	21 (100)	20 (100)	16 (100)	7 (100)	4 (100)
	I	0	0	0	0	0	0	0	0	0
	R	0	0	9 (50.0)	17 (50.0)	0	0	0	0	0
Erythromycin (0.016–256 mg/l)	S	8 (100)	5 (100)	15 (83.3)	0	21 (100)	20 (100)	5 (31.3)	4 (57.1)	1 (25.0)
	I	0	0	0	10 (29.4)	0	0	0	0	0
	R	0	0	3 (16.7)	24 (70.6)	0	0	11 (68.8)	3 (42.9)	3 (75)
Colistin (0.064–1024 mg/l)	S	8 (100)	5 (100)	18 (100)	34 (100)	6 (28.6)	5 (25.0)	0	0	4 (100)
	I	0	0	0	0	0	0	4 (25)	0	0
	R	0	0	0	0	15 (71.4)	15 (75)	12 (75)	7 (100)	0
Chloramphenicol (0.016–256 mg/l)	S	8 (100)	3 (60.0)	18 (100)	11 (32.4)	16 (76.2)	17 (85.0)	14 (87.5)	2 (28.6)	4 (100)
	I	0	2 (40)	0	0	0	0	0	0	0
	R	0	0	0	23 (67.6)	5 (23.8)	3 (15.0)	2 (12.5)	5 (71.4)	0
Trimethoprim/ sulfamethoxazole (0.002–32 mg/l)	S	8 (100)	5 (100)	18 (100)	14 (41.2)	18 (85.7)	20 (100)	16 (100)	7 (100)	4 (100)
	I	0	0	0	0	3 (14.3)	0	0	0	0
	R	0	0	0	20 (58.8)	0	0	0	0	0
Gentamicin (0.002–32 mg/l)	S	5 (62.5)	5 (100)	18 (100)	23 (67.6)	14 (66.7)	16 (80.0)	13 (81.2)	4 (57.1)	4 (100)
	I	0	0	0	0	0	4 (20.0)	0	0	0
	R	3 (37.5)	0	0	11 (32.4)	7 (33.3)	0	3 (18.8)	3 (42.9)	0
Rifampicin (0.002–32 mg/l)	S	0	0	6 (34.1)	0	21 (100)	20 (100)	6 (37.5)	3 (42.9)	0
	I	0	0	3 (10.6)	0	0	0	3 (18.8)	0	0
	R	8 (100)	5 (100)	9 (50.0)	34 (100)	0	0	7 (43.8)	4 (57.1)	4 (100)

Nalidixic acid	S	6 (75.0)	4 (80.0)	13 (72.2)	0	0	0	0	0	4 (100)
(0.016–256 mg/l)	I	0	0	0	4 (11.8)	0	0	4 (25)	0	0
	R	2 (25.0)	1 (20.0)	5 (27.8)	30 (88.2)	21 (100)	20 (100)	12 (75.0)	7 (100)	0
Ciprofloxacin	S	5 (62.5)	5 (100)	18 (100)	15 (44.1)	17 (81.0)	16 (80.0)	5 (31.3)	5 (71.4)	4 (100)
(0.002–32 mg/l)	I	0	0	0	0	4 (19.0)	0	0	0	0
	R	3 (37.5)	0	0	19 (55.9)	0	4 (20.0)	11 (68.8)	2 (28.6)	0
Penicillin G	S	0	0	8 (44.4)	0	19 (90.5)	17 (85.0)	13 (81.2)	6 (85.7)	0
(0.002–32 mg/l)	I	0	0	0	0	0	0	0	0	0
	R	8 (100)	5 (100)	10 (55.6)	34 (100)	2 (9.5)	3 (15.0)	3 (18.8)	1 (14.3)	4 (100)
Ampicillin	S	3 (67.7)	3 (60.0)	14 (77.8)	14 (41.2)	17 (81.0)	16 (80.0)	9 (56.3)	5 (71.4)	3 (75.0)
(0.016–256 mg/l)	I	0	0	0	0	4 (19)	0	3 (18.8)	0	0
	R	5 (37.5)	2 (40.0)	4 (22.2)	20 (58.8)	0	4 (20.0)	4 (25.0)	2 (28.6)	1 (25.0)
Imipenem	S	8 (100)	5 (100)	18 (100)	34 (100)	18 (85.7)	20 (100)	12 (75.0)	7 (100)	4 (100)
(0.002–32 mg/l)	I	0	0	0	0	3 (14.3)	0	0	0	0
	R	0	0	0	0	0	0	4 (25.0)	0	0
Cefalotin	S	2 (25.0)	0	13 (72.2)	9 (26.5)	16 (76.2)	18 (90.0)	11 (68.8)	4 (57.1)	3 (75.0)
(0.016–256 mg/l)	I	0	1 (20.0)	0	5 (14.7)	0	0	0	0	0
	R	6 (75.0)	4 (80.0)	5 (27.8)	20 (58.8)	5 (23.8)	2 (10.0)	5 (31.2)	3 (42.9)	1 (25.0)
Ceftriaxone	S	5 (62.5)	1 (20.0)	16 (88.9)	0	12 (57.1)	15 (75.0)	6 (37.5)	5 (71.4)	4 (100)
(0.016–256 mg/l)	I	0	0	2 (11.1)	0	0	0	0	0	0
	R	3 (13.5)	4 (80.0)	0	34 (100)	9 (42.9)	5 (25.0)	10 (62.5)	2 (28.6)	0
Teicoplanin	S	0	0	0	0	0	3 (15)			
(0.016–256 mg/l)	I	0	0	0	0	13 (61.9)	0	0	0	0
	R	8 (100)	5 (100)	18 (100)	34 (100)	8 (38.1)	17 (85.0)	16 (100)	7 (100)	4 (100)
Vancomycin	S	0	0	0	0	9 (42.9)	9 (45.0)	0	0	0
(0.016–256 mg/l)	I	0	0	0	0	3 (14.3)	5 (25.0)	0	0	0
	R	8 (100)	5 (100)	18 (100)	34 (100)	6 (28.6)	6 (30.0)	16 (100)	7 (100)	4 (100)

[a]: N (%); S: Suseptible; I: Intermediately Susceptible; and R: Resistance.

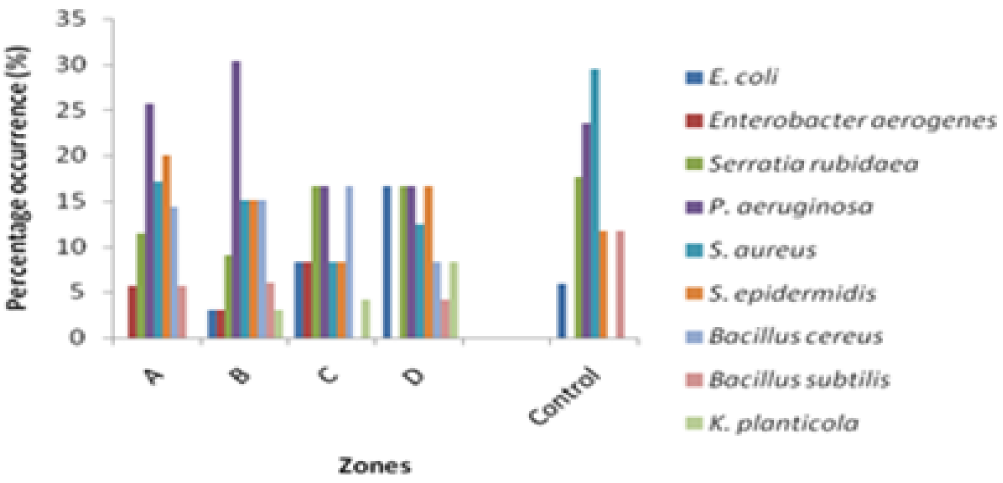

Figure 1. The occurrence percentage of individual bacterial species in suya spices from different zones in Ogun State, Nigeria.

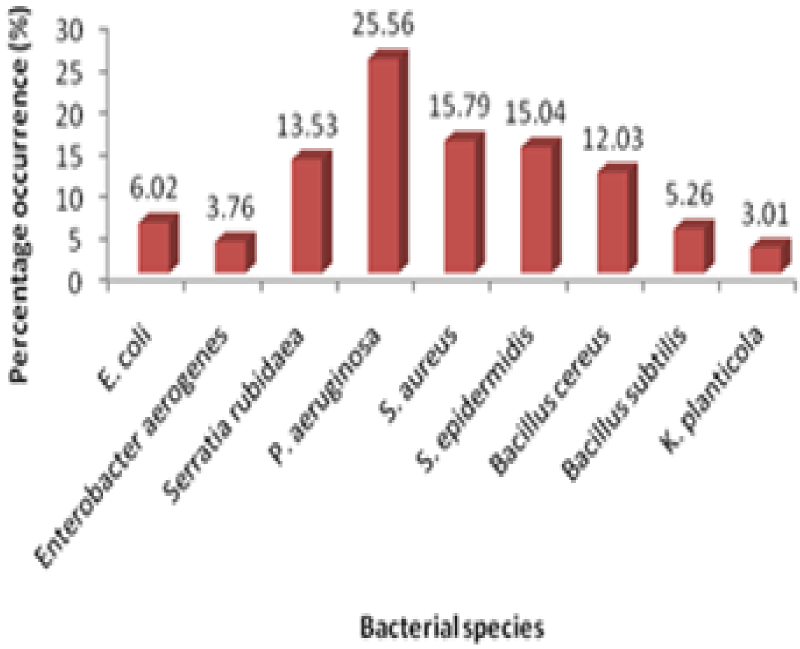

Figure 2. The occurrence percentage of the bacterial species from suya spices.

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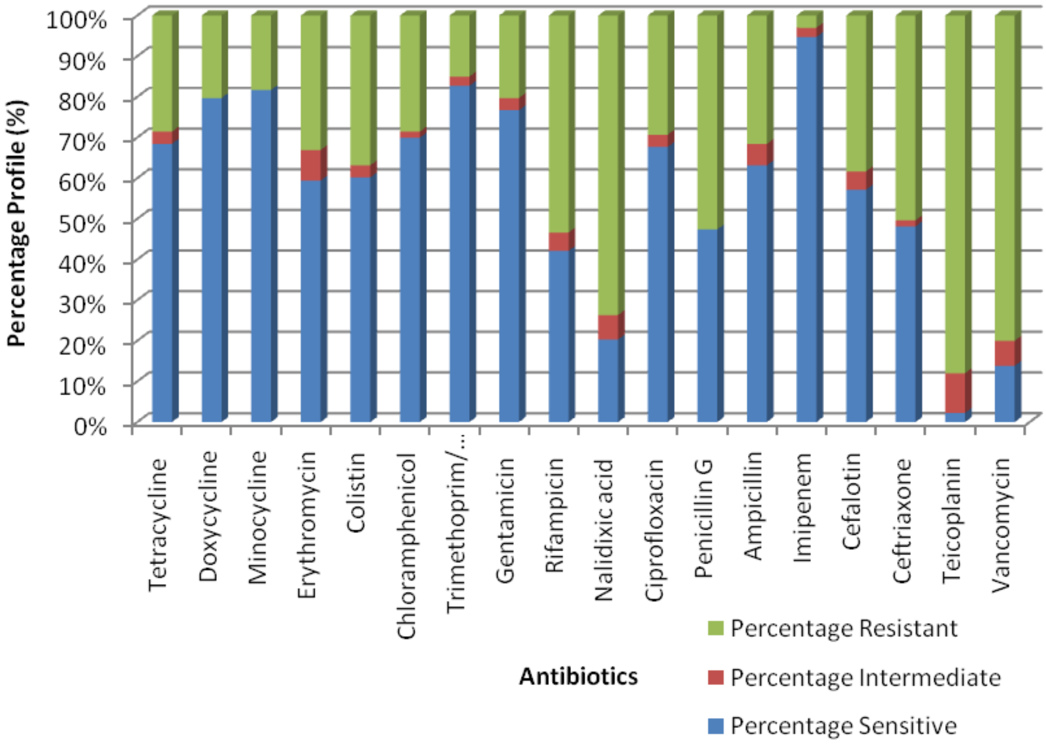

Figure 3. Cumulative percentage susceptibility and resistance profile of bacterial isolates from suya spices in Ogun State, Nigeria.

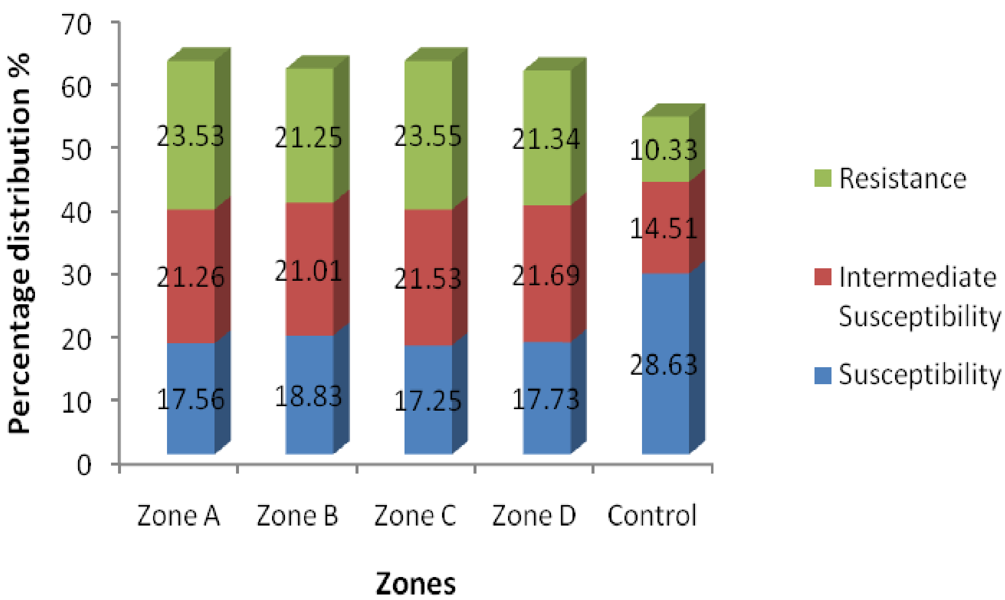

Figure 4. Cumulative distribution of susceptible and resistant bacterial strains from suya spices in different zones of Ogun State, Nigeria. The data are not statistically significant at 95% level of confidence i.e. $P > 0.05$.

Discussion

This study indicated that most suya vendors purchased spices, such as curry powder, paprika, pepper, ginger, nutmeg, chilli pepper (all in powdered form) from the market which were then mixed in a plastic container. However, it was noted that these vendors mixed the spices with their bare hands without wearing hand gloves and the mixed spices were left uncovered in the plastic container. Therefore, the spices are exposed to contaminants, such as dust, atmospheric particles, and airborne microbes. Many food vendors are faced with the challenges of lack of public infrastructures to comply with standard hygienic practices, such as clean water sources, public toilets, inadequate waste disposal service, poor sanitary conditions at vending sites (for example dust from dirt roads, open-air sewages, traffic fumes), poor storage conditions, contaminated inputs from farmers and market sellers, and unclean transportation conditions. El-Hassan *et al*. reported that spices, being cultivated and collected in tropical areas using traditional methods, were usually exposed to contaminants from the soil and air, before being well-dried to prevent possible microbial growth, as well as during harvesting, handling, and packing (El-Hassan *et al.*, 2018).

Generally, total viable bacterial count, in spices, equal to or more than 6 log cfug^{-1} is not acceptable based on the International Commission on Microbiological Specification for Food (Van Schothorst *et al.*, 2011). The mean TPCs of suya spices in this study (**Table 1**) were lower than the bacterial counts obtained from suya spice ingredients and in spicy meat products (Amala and Onwuli, 2017, El-Hassan *et al.*, 2018). However, the isolation of potential pathogens from spices is in agreement with the reports of (Bakobie *et al.*, 2017, Odu and Akwasiam, 2016). The presence of potential pathogens indicated bacterial contamination of the suya spice ingredients. All bacterial species discovered in this study were found in zones A, B, C, and D in different proportions (**Figure 1** and **Figure 2**). The cumulative frequency of bacterial species showed that *P. aeruginosa* had the highest frequency, followed by *S. aureus*, while the least was *K. planticola* (**Figure 2**). In a similar study, *S. aureus* was reported as the most frequent organism, followed by *E. coli* and then *Salmonella* spp (Odu and Akwasiam, 2016). Amare *et al.* also reported *S. aureus* (53.96%) as the most frequent isolate, followed by *E. coli* (23.8%), *Enterobacter* species (15.87%), and *Citrobacter* species (6.3%) (Amare *et al.*, 2019).

The poor unhygienic practices and improper storage of suya spice ingredients might have contributed to the level of contamination recorded. Statistical analysis showed significant difference in mean count of bacteria isolated from suya spice ingredients obtained from the different zones compared to the control (P < 0.05). Some reports have suggested that the preparation processes of suya and suya spices were usually carried out under unhygienic conditions, indicating a high risk of contamination (Amala and Onwuli, 2017, Bello Temitope and Bello Olorunjuwon, 2020).

Spice ingredients could possibly get contaminated from the farm and, more especially, during transportation from a place to another. Non-sterile state of equipment and poor education of most vendors could be linked with contamination of the spices with the potential pathogens. Poor management of market environment has also been identified as a potential source of contamination, in addition to poor storage system. These conditions make many food products vulnerable and expose consumers to the risk of pathogenic bacteria invasion and food poisoning. Statistically, occurrence of bacteria in the samples from the sites was not significantly different (P > 0.05). Hence, source of contamination of the potential pathogens differed, depicting the multiple sources of contamination of the spices. Spice ingredient contamination can, therefore, be prevented if aseptic techniques are embraced from the farms, through preparatory stages, to the table.

Staphylococcus is a spherical, Gram- and catalase-positive, non-motile, non-spore forming bacterium that appears in pairs, short chains or grape-like clusters under the microscope. This facultative aero-anaerobic bacterium is cosmopolitan in nature and, thus, could be found in/on humans and animals, in air, dust, sewage, water, and on environmental surfaces (Bello *et al.*, 2013). *S. aureus* is a major cause of staphylococcal food poisoning and one of the most common foodborne outbreaks around the world as a result of the ingestion of staphylococcal enterotoxins produced by the enterotoxigenic strains of coagulase-positive staphylococci and, in rare cases, by other staphylococci species, such as *S. intermedius* (Hennekinne *et al.*, 2012). *S. epidermidis* is associated with the human epithelia. The bacterium differs from coagulase-positive staphylococci, such as *S. aureus* by lacking the enzyme coagulase. *S. epidermidis* is responsible for most infections, among the coagulase-negative staphylococci (CoNS) (Namvar *et al.*, 2014). The bacterium causes infections associated with any type of indwelling medical devices, including peripheral or central intravenous catheters (CVCs) (Otto, 2009).

B. cereus occurs as the most frequent aerobic spore bearer in many types of soil, in sediments, dust, and plants. It is also commonly found in food production environments as a result of the adhesive nature of its endospores, enabling it to spread to most kinds of food (McDowell *et al.*, 2020). *B. cereus* thrives on decaying organic matter, vegetables and fomites, in fresh and marine waters, and the intestinal tract of invertebrates, through which soil and food products often get contaminated (Bottone, 2010). *B. subtilis* has the ability to persist in the environment for a long period of time. The bacterium is often regarded as non-pathogenic. It has, however, been reported to be associated with food poisoning as a result poor quality bakery products among others. The food poisoning caused by *B. subtilis* has a rapid onset, with acute vomiting, and commonly accompanied by diarrhea (Gu *et al.*, 2019).

Enterobacter belongs to a genus of rod-shaped, Gram-negative, non-spore-forming, facultative anaerobic bacteria in the family *Enterobacteriaceae*. *E. aerogenes* is one of the two well-known species (the second being *E. cloacae*) and has taken on clinical significance as opportunistic bacteria. It has also been implicated as a nosocomial pathogen from intensive care patients pathogenic, particularly to those who are on mechanical ventilation (Khan *et al.*, 2017). *E. aerogenes* is widely distributed in soil, water, sewage, vegetables, and dairy products. Enterobacter species exhibit resistance to most antibiotics, including the cephalosporins (Peirano *et al.*, 2018).

P. aeruginosa is ubiquitous in nature and involves in several interactions with eukaryotic host organisms. It is an opportunistic pathogen in humans and causes a wide range of infections in community and healthcare settings (Benie *et al.*, 2017). It is frequently associated with infections, including bacteraemia, pneumonia, urinary tract infections, and wound infections (Bello *et al.*, 2018). *P. aeruginosa* was reported as the 2nd most frequent organism associated with ventilator-associated pneumonia, the 4th most common organism associated with catheter-associated urinary tract infections, the 5th major agent that causes surgical site infections, and the 7th associated with central-line-associated bloodstream infections (Mansour *et al.*, 2013). This organism has shown resistance to many antibiotics (Benie *et al.*, 2017, Mansour *et al.*, 2013).

E. coli is popular as the main commensal inhabitant of gastrointestinal tract of mammals. It is, however, responsible for several infections in both humans and animals. The pathogenic strains are associated with several types of human diarrhea (Ahmed *et al.*, 2021). Infections caused by different pathotypes of *E. coli* are of tremendous public health concern. The presence of this enteric organism in suya spices is indicative of contamination with matter of faecal origin. Infections of humans with *Serratia* are not as common as with more virulent members of the Enterobacteriaceae. *Serratia rubidaea* is a less well-described member of the genus and is commonly present in water, soil, and food. The isolation of this organism from food samples is not common, but has been reported to cause opportunistic infections (Litterio *et al.*, 2012, Yao *et al.*, 2016). The organism can develop increased resistance to many antibiotics.

A high likelihood of therapeutic success could be linked with the concentration of an antibiotic that is capable of inhibiting a bacterial strain *in vitro* and the bacterial strain is said to be susceptible to the antibiotic. When a bacterial strain is inhibited *in vitro* by a certain concentration of an antibiotic with an uncertain therapeutic effect, the sensitivity of the bacterial is said to be intermediate. A resistant bacterial strain to a certain antibiotic is inhibited *in vitro* by a concentration of the antibiotic and which is associated with a high therapeutic failure.

The bacterial isolates displayed varying resistance pattern to different antibiotics investigated in the study (Table 3). Imipenem exerted potency against 94.74% the bacterial isolates making the antibiotic the most potent in this study. Only 3.01% of the isolates exhibited resistance to imipenem, while up to 87.97% of isolates showed resistance to teicoplanin (Fig. 3). There was no statistical difference ($p > 0.05$) in resistance profiles of bacterial isolates from the four zones ($p > 0.05$). There was, however, a significant difference between the control and the four zones ($p < 0.05$) (Fig. 4). This depicts that home-prepared suya spices, under controlled condition, had the best microbial quality. It also confirmed the likely factors earlier mentioned to be associated with contamination of suya spices in this study.

The findings on the antibiotic resistance of bacteria in this study were in line with the results of (Amare *et al.*, 2019) who reported that ciprofloxacin was one of the most effective antibiotics against *S. aureus* isolates. (Sani *et al.*, 2012) also stated that *S. aureus* was sensitive to the fluoroquinolones. (Amadi *et al.*, 2016) demonstrated that ciprofloxacin showed high sensitivity and broad-spectrum activity to similar organisms investigated in their study, which is consistent with the position of this study. Ciprofloxacin belongs to the fluoroquinolone class of antibiotics, and this class is known to possess excellent activities against Gram-negative and Gram-positive bacteria (Cohen *et al.*, 2016). However, a high percentage of the isolates exhibited resistance to nalidixic acid. The fluoroquinolones are known to inhibit the bacterial DNA gyrase that enables DNA replication and transportation (Moore D, 2015). Ampicillin also inhibited the growth of 63.12% of the bacterial isolates, which contradicts the results of (Amare *et al.*, 2019) who reported poor activities of ampicillin against similar organisms.

Exactly 13.53% and 2.26% of the bacterial isolates in this study showed sensitivity to vancomycin and teicoplanin, respectively. This could be attributed to the narrow-spectrum activity of the antibiotics and their weak actions against many Gram-negative bacteria. Vancomycin and teicoplanin are glycopeptides, whose modes of action are similar to the β-lactam antibiotics. However,glycopeptides interfere with different molecular targets as they bind to acyl-D-alanyl-D-alanine in peptidoglycan and, thus, prevent the function of glycosyltranferases in the susceptible bacteria. The β-lactams, which are hydrophilic, pass through porins, and glycopeptides lack the ability to cross the outer membrane due to their structures, preventing the use of any of these passages (Bello Temitope and Bello Olorunjuwon, 2020, Breijyeh *et al.*, 2020).

Cefalotin and ceftriaxone belong to the class cephalosporins (first and third generations, respectively). The mechanism of acion of the cephalosporins is similar to that of penicillin-based antibiotics. The antibiotics in this category disrupt the peptidoglycan layer of bacterial cell walls by inhibiting the penicillin-binding proteins (Moore D, 2015). Exactly 57.14% and 48.12% of the bacterial were sensitive to cefalotin and ceftriaxone, respectively. The relatively weak activity of these antibiotics suggests that many of the resistant isolates could contain the enzyme β-lactamase, deactivating the β-lactam rings of the β-lactam antibiotics and cephalosporins. This is buttressed by the findings of Bello et al. (2019) who reported the resistance profile of β-lactamase-producing bacterial isolates from salad vegetables (Bello *et al.*, 2019).

The growth of exactly 82.70% and 60.15% of the bacterial isolates were inhibited by trimethoprim/sulfamethoxazole and colistin, respectively, while 54.90% of the bacterial isolates were sensitive to erythromycin. This result is in line with the report of (Hardman *et al.*, 2017) who reported that over half of the studied organisms were inhibited by the same class of antibiotics. Erythromycin is a macrolide-based antibiotic and the mechanism of action is the inhibition of protein synthesis by reversibly binding to the 50s ribosomal subunit (Moore D, 2015).

Gentamicin is an aminoglycoside and this class of antibiotics bind irreversibly to the 16S rRNA subunit of the 30S ribosome, leading to the inhibition of bacterial protein synthesis. Gentamicin inhibited 76.69% of the bacterial isolates and this high potency could be connected with the mechanism of action of this class of antibiotics. This finding is supported by the studies of (Barber *et al.*, 2018, Breijyeh *et al.*, 2020, Mhondoro *et al.*, 2019). In this study, 87.50% of the isolates were sensitive to chloramphenicol, belonging to the phenicol class of antibiotics and whose mechanism of action is to disrupt protein sysnthesis in bacteria. Resistance of bacterial isolates to chloramphenicol could be associated with the production of chloramphenicol acetyltransferase (CAT), while some resistance is usually as a result of inability of certain bacteria to reach their target sites.

Minocycline, doxycycline, and tetracycline exerted effiicacy on 87.21%, 79.70%, and 68.42% of the isolates, respectively. These antibiotics belong to the class - tetracylines - which inhibit the synthesis of protein by blocking the adherence of aminoacyl-tRNA to the ribosomal acceptor (A) site. Their high potency displayed could not be dissociated from the fact that they are broad-spectrum antibiotics. However, a high resistance profile to this class of antibiotics was reported by (Mhondoro *et al.*, 2019). Imipenem, a penicillin-based antibiotic, acts by binding to and inactivating penicillin-binding proteins (PBPs) situated on the inner membrane of bacterial cell wall. The rigidity and strength of the bacterial cell wall are interrupted by PBPs inactivation, which interferes with the cross-linkage of peptidoglycan chains. It prevents the synthesis of bacterial cell wall and weakens it while leading to cell lysis (Niwa *et al.*, 2016).

Conclusion

This study reveals low bacterial load but the presence of potential pathogens in suya spices. This depicts that suya spices, when added to suya meat, are one of the major sources of potential pathogens in ready-to-eat suya meat preparation, and which could lead to the dissemination of resistant pathogens. Therefore, it becomes imperative to enlighten suya vendors and other food handlers on the significance of hygiene practices from farm to kitchen. Suya spices should be properly covered to prevent post- and cross-processing contaminations by microbial pathogens. Intensive surveillance and monitoring of roasted and vended foods should be put in place to enhance microbial safety of the food product. Federal and State Food Regulatory Authorities should be responsible in discharging their statutory duties to ascertain that the foods getting to the consumers' tables are safe, wholesome, and of standard quality.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Authors' contributions

This study was carried out in collaboration between both authors. Both authors designed the study. Author O.O. Bello wrote the first draft of the manuscript and managed the literature review. Author T.K. Bello assisted in the collection, preparation, and transportation of samples. Both authors managed the analyses of the study, read and approved the final manuscript.

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